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Laboratory Methods for Ten Hepatic Toxification/Detoxification Parameters

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16. ABSTRACT

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JOURNAL ARTICLE

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Kirk T. Kitchin

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Key words: Hepatic Drug Metabolizing Enzymes, Toxification Reactions, Detoxification Reactions, Cytochrome P-450, NADPH-Cytochrome C Reductase, Glutathione, Glutathione S-transferase, UDP-glucuronyltransferase.

Introduction

The mportance of drug metabolizing enzymes of the liver and other organs to pharmacology and toxicology has been well described in several review papers (1-8). Collectively, biotransformation (sometimes called toxification) and conjugation (or detoxification) reactions modify various functional groups, increase the polarity of parent compounds, and often hasten renal or biliary excretion. Understanding biotransformation and conjugation is made more difficult by the presence of multiple forms of enzymes such as cytochrome P-450 (9-13), glutathione S-transferase (5, 6, 14), and UDP-glucuronyltransferase (7, 8). Chemical treatments may affect enzyme activities by induction, repression, inhibition, cofactor depletion, and inactivation. The major enzymatic elements of hepatic toxification/detoxification and their relationship to one another is illustrated in Figure 1.

In this review article, biological background information and specific methods for 10 hepatic toxification/detoxification parameters are presented. All enzymatic methods have been verified in the author's laboratory to be linear with time and protein concentration and to utilize saturating substrate and cofactor concentrations. When methods are particularly well described in published literature, references are prominently given in the text. If the assay can be run on frozen and thawed samples without loss of enzyme activity or tissue content, this is explicitly stated. Otherwise the enzyme assay should be performed quickly, using freshly prepared hepatic subcellular

US Environmental Protection Agency, Health Effects Research Laboratory, Experimental Biology Division, Cellular Biophysics Branch, Research Triangle Park, NC 27711.
Send all correspondence to: Dr. Kirk T. Kitchin, MD-74. Experimental Biology Division, HERL. EPA Research Triangle Park, NC 27711.

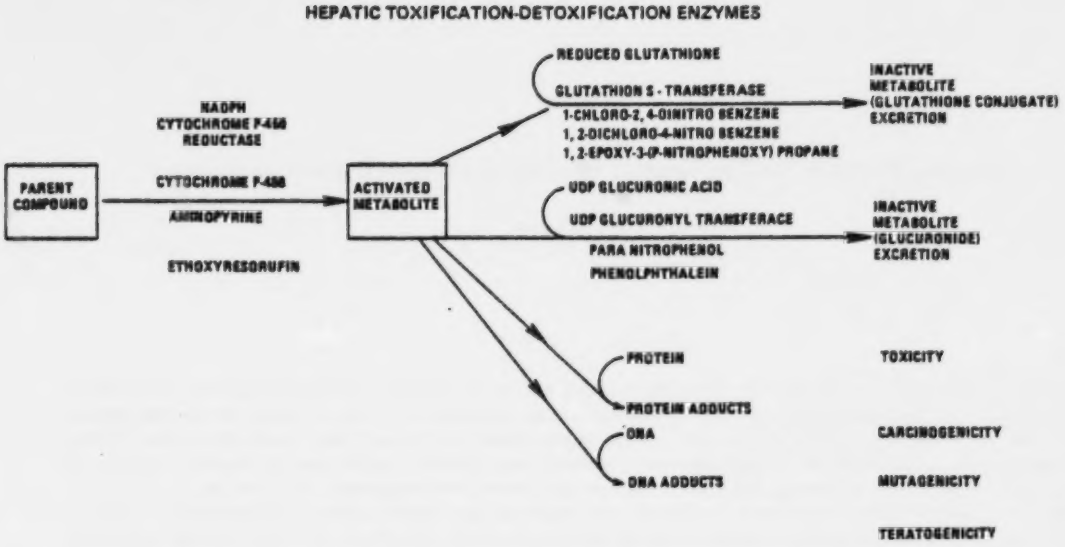

Fig. 1. Parent compounds are metabolized via the cytochrome P-450 monooxygenase system. Representative substrate aminopyrine and ethoxyresorufin can be used to assay the in vitro activity of this oxidative enzyme system. Metabolites may be detoxified via the glutathione-S-transferase system or the glucuronidation system. Representative substrates for these two enzymes are written below the enzyme name while cofactors are written just above it. Alternatively an activated metabolite may form adducts with important tissue macromolecules such as protein or DNA. This covalent binding can lead to toxicity, carcinogenicity, mutagenicity or teratogenicity. (adapted from 42)

fractions (high speed supernatant or microsomes). Investigators should *always* check to make sure the enzyme assays they perform are linear with time and protein concentration with tissue preparation from their experimental animals in their laboratory. The protein and time assay conditions given as a guide in this review should not be used for other strain rats or different species without first verifying that all the assay conditions are appropriate.

Background information and assay method for ten hepatic toxification detoxification parameters

NADPH-cytochrome C reductase (NADPH-cytochrome P-450 reductase)

The cytochrome P-450 dependent monooxygenase system depends on a supply of electrons obtained from NADPH or NADH via the flavoprotein NADPH-cytochrome P-450 reductase. Depending on the electron acceptor used, this enzyme activity is called NADPH-cytochrome C or NADPH-cytochrome P-450 reductase. The *in vivo* microsomal electron acceptor is cytochrome P-450 of course, but the assay of reductase activity is far easier to perform with cytochrome C as the substrate.

The NADPH-cytochrome P-450 reductase system is inducible up to 2-3 fold. Some investigators have hypothesized the activity of this reductase enzyme may limit cytochrome P-450 based enzyme activity (15). Therefore, coordinate induction of NADPH-cytochrome P-450 reductase activity along with cytochrome P-450 becomes important in assessing the overall rates of oxidative biotransformation. In studies with the reconstituted microsomal monooxygenase system either the lipid, hemoprotein or reductase component can be made rate limiting (16). Higher rates of cytochrome P-450 based catalysis (15-17) are not always obtained from *in vitro* incorporation of exogenous cytochrome P-450 into microsomal membranes.

Table I. Assay Conditions for Eight Hepatic Toxification/Detoxification Enzyme Activities

Enzyme	NADPH cytochrome C reductase	Aminopyrine N-demethylase	Ethoxyresorufin O-deethylase	Glutathione S-transferase (CDNB)	Glutathione S-transferase (DCNB)	Glutathione S-transferase (ENPP)	UDP Glucuronyl transferase (p-nitrophenol)	UDP Glucuronyl transferase (phenolphthalein)
Substrate	cytochrome C	aminopyrine	ethoxyresorufin	1-chloro-2,4-dinitrobenzene	1,2,dichloro-4-nitrobenzene	1,2-epoxy-3-(p-nitro-phenoxy) propane	p-nitrophenol	phenolphthalein
Substrate Concentration (mM)	0.034 (0.43 mg/ml)	1.8	0.00025 (0.25 μM)	1.0	1.0	0.8	0.8	0.2
Cofactor(s), Inhibitor	NADPH, KCN	NADP*, Glucose 6 Phosphate, Glucose 6 Phosphate dehydrogenase	NADPH (or generating system	Glutathione	Glutathione	Glutathione	UDP-glucuronic acid	UDP-glucuronic acid
Cofactor(s) Concentration (mM)	1.5, 1.2	0.3, 10.0, 1.0 units/ml	1.0	1.0	5.0	5.0	1.4	1.4
Buffer	0.05M Tris-HCl	0.05M Tris HCl W/ 0.0152M $MgCl_2$	0.1M KP	0.1M KP	0.1M KP	0.1M KP	0.05M Tris-HCl W/ 0.01M $MgCl_2$	0.5M Tris-HCl W/ 0.01M $MgCl_2$
Buffer pH	7.5	7.5	7.8	6.5	7.5	6.5	7.4	7.4
Type of Assay	kinetic	end point	end point (or kinetic)	kinetic	kinetic	kinetic	end point	end point
Tissue Storage	frozen	fresh	fresh	frozen	frozen	frozen	frozen	frozen
Protein Linearity (mg/ml)	0.03	2.20	0.20	0.003	0.048	0.12	0.75 0.2% Triton X-100	0.75 0.2% Triton X-100
Time Linearity (min)	2	10	10	2	4	5	10	10
Blanks	omit NADPH	Add 10% Trichloro-acetic acid	omit NADPH	omit protein	omit protein	omit protein	omit UDPGA	omit UDPGA
Start Reaction	substrate	protein	substrate	substrate	substrate	substrate	protein	protein
Stop Reaction	–	10% trichloro-acetic acid	cold acetone	–	–	–	14 ml of 0.2 M glycine buffer pH 10.4	7 ml of 0.2 ml glycine buffer pH 10.4
Wavelength (nm)	550	406	excitation 510 emission 585	340	346	360	405	553
ε Coefficient of product or substrate mM^{-1} cm^{-1}	19.1	8.0	–	9.6	10.0	0.5	14.5	31.4
Absorption Scale	0.5	1.0	–	1.0	0.5	0.2	1.0	1.0
Reference	18	22, 23, 24	20, 31, 32	14	14	14	41	40

This result would be expected if the NADPH-cytochrome P-450 reductase component of the monooxygenase system was rate limiting.

The kinetic assay of NADPH cytochrome C reductase is performed at 37° C at 550 nm, using cuvettes stirrers if available (18). The final concentrations of the substrate, cofactors and microsomal protein are as listed in Table I. Stock solutions of cytochrome C (18 mg/5 ml) and KCN (4.8 mg/5 ml) in the assay buffer can be made up in advance and stored frozen. Aliquots of 0.3 and 0.2 ml of cytochrome C and KCN respectively will give the final required assay concentrations in a final total cuvette volume of 2.5 ml. NADPH solutions should be prepared on the day of assay. Once a stable baseline is obtained, the kinetic reaction is initiated by the addition of the highly colored substrate cytochrome C. Blanks without NADPH should be used. This assay is among the easiest of the hepatic toxification/detoxification procedures to perform. Little protein is required, only short time courses are needed and the hepatic microsomal preparations can be stored frozen. A millimolar extinction coefficient of 19.1 can be used for the difference in light absorption at 550 nm for reduced minus oxidized cytochrome C.

Cytochrome P-450

This versatile hemoprotein is found in most mammal organs. At least 6 forms of this protein exist in rodent liver (9-13). The 2 main forms are often called cytochrome P-450 and cytochrome P-448 and refer to the phenobarbital or 3-methylcholanthrence inducible forms, respectively. Spectral, enzymatic and biochemical methods exist to differentiate between various cytochrome P-450

forms. The representative substrates aminopyrine (P-450) and ethyoxyresorufin or benzo[α]pyrene (P-448) can be used as markers for these different rat hemoproteins (19, 20). Phenobarbital administration increases liver weight, microsomal protein, NADPH-cytochrome C reductase, cytochrome P-450 content and its associated activities, and some glutathione-S-transferase activities. In rats the reduced carbon monoxide difference spectra of cytochrome P-450 induced by phenobarbital is very similar to the endogenous cytochrome P-450.

3-Methylcholanthrene has more specific inductive effects. Cytochrome P-450 of different spectral characteristics (*e.g.,* the carbon monoxide reduced difference spectral absorption maximum occurs at 448 nm) is synthesized and enzyme activities like ethoxyresorufin O-deethylase and benzo[α]pyrene hydroxylase (aryl hydrocarbon hydroxylase) are strongly induced. With respect to the rat hepatic cytochrome P-450 system, phenobarbital and 3-methylcholanthrene induction do not overlap to a significant extent. In general, most cytochrome P-450 inducers are somewhat planar lipid soluble organic molecules and they may be substrates of the cytochrome P-450 system as well.

The cytochrome P-450 assay requires either a dual beam or a dual wavelength spectrophotometer because of the high light absorption and scattering of the microsomal suspension. It is faster to perform this assay with a dual wavelength spectrophotometer. Storage and use of the highly toxic gas carbon monoxide warrants appropriate laboratory safety measures. Sodium dithionite should be stored desiccated in small separate containers as the assay may occasionally fail because the dithionite has become oxidized.

The method of Omura and Sato (21) is universally used to determine the amount of cytochrome P-450 present in subcellular fractions. A spectral baseline from 400 to 500 nm of carbon monoxide-exposed hepatic microsomes should be run to make sure the microsomal sample is evenly distributed between the sample and reference cuvettes in a dual beam spectrophotometer. When complexed with carbon monoxide, the sodium dithionite reduced minus oxidized cytochrome P-450 gives a prominent peak at approximately 450 nm. The difference in light absorption at 450-490 nm is used along with an extinction coefficient of 91 mM^{-1} cm^{-1} to determine the cytochrome P-450 level (21).

Cytochrome b_5 is the other heme containing component of rat liver microsomes. As NADH will selectively reduce cytochrome b_5 and not cytochrome P-450, these two hemoproteins may be sequentially assayed in the same microsomal suspension. To do so, first record a dual beam baseline from 400 to 500 nm. Second, add 100 ul of 10 mg/ml solution of NADH to the sample cuvette (add 100 μl of buffer to the reference cuvette) and mix well. After recording the NADH-reduced difference spectra from 400-500 nm, the cytochrome b_5 level can be calculated by using the extinction coefficient of 185 mM^{-1} cm^{-1} for the absorption difference between 424 and 410 nm (21). Now reduce the reference cuvette with dithionite (both cytochrome P-450 and b_5 are reduced by dithionite), bubble CO for 15 seconds through both cuvettes and reverse the original cuvettes' positions. After scanning from 400-500 nm, the difference in light absorption between 450 and 490 nm is used to quantitate cytochrome P-450 level via the normal procedure. All the procedures can be done at room temperature, but the microsomal preparations should be kept on ice prior to assay.

Aminopyrine N-demethylase

This enzyme activity depends on all the components of the monooxygenase system: cytochrome P-450, NADPH-cytochrome P-450 reductase and microsomal lipid. This particular cytochrome P-450 dependent enzyme activity is an end point assay done at 37° C with a shaking water bath in open air. Nash (22) originally described the method used to determine formaldehyde, the end product of the reaction. The assay described here is based on the procedure given by Lucier et al. (23). In 0.05 M Tris-HCL Buffer, pH 7.5, containing 15.6 mM $MgCl_2$, the enzyme reaction is run using a NADPH generating system. The NADPH generating system consists of final con-

concentrations of 0.4 $NADP^+$, 10 mM glucose 6-phosphate and 1 unit/ml of glucose 6-phosphate dehydrogenase. The glucose-6-phosphate can be prepared in advance and stored frozen; the glucose 6-phosphate dehydrogenase can be stored for several weeks at refrigerator temperatures, but it is recommended that the $NADP^+$ stock solution be prepared fresh on the day of use.

For blanks, 1.0 ml of 10% trichloroacetic acid is added before the microsomes. The substrate concentration should be 1.8 mM and the protein concentration can be quite high, up to 2.2 mg microsomal protein per ml. After the ten minute 37° assay is terminated by addition of 1.0 ml of 10% trichloroacetic acid to the 30 ml corex centrifuge tubes in which the enzyme assays are run, the test tubes are spun at 9,000 x g for 10 min. To 2 ml of the supernatant, 1 ml of Nash Reagent (154 g of ammonium acetate, 3 ml of glacial acetic acid and 2 ml of acetylacetone in 1 liter of water) is added (22). The mixture is heated at 60° C for 8 minutes in test tubes covered with parafilm and then cooled at room temperature for 15 minutes. The resulting light absorption is read at 405 nm in a spectrophotometer. Internal standards of formaldehyde should be used to establish a standard curve.

Controversy exists in the literature as to the possible need for semicarbazide to trap formaldehyde before the derivative formation step. In our laboratory this has proved to be unnecessary. However, semicarbazide can be added just after the 10% trichloraacetic acid termination step without damage to the assay. If it is added before the enzyme assay is performed, inhibition of aminopyrine N-demethylase may occur (24).

A radiometric aminopyrine N-demethylase assay has been described by Poland and Nebert (25). This highly sensitive assay is good for certain applications, particularly some extrahepatic tissues, where extreme sensitivity is needed. The sensitivity of the radiometric assay depends on prior purification of the substrate by a simple extraction procedure. Normally the colorometric assay is sufficiently sensitive for liver preparations.

Ethoxyresorufin O-deethylase

Benzo[α]pyrene metabolism is often used as a measure of cytochrome P-448 mediated enzyme activity. The two main reasons for this are the interest in benzo[α]pyrene as a model polycyclic hydrocarbon carcinogen and the extreme sensitivity the fluorometric version of the benzo[α]pyrene assay offers. The chief disadvantages are the additional safety precautions that are required by utilizing a carcinogenic substrate and the complex metabolic profile of benzo[α]pyrene. Many metabolites of benzo[α]pyrene can presently be identified (26, 27). The fluorometric assay chiefly detects only 3-hydroxybenzo[α]pyrene, while radiometric assays of benzo[α]pyrene metabolism can detect all polar metabolites (28, 29).

For reasons of laboratory safety and simplicity of metabolism (26, 27, 30), the O-deethylation of ethoxyresorufin is an excellent choice for a measure of rat hepatic microsomal cytochrome P-448 dependent metabolism. This assay is well described by Burke and Mayer (20, 31, 32). It requires a spectrophotofluorometer and can be done kinetically or as an end point reaction. Ten minute shaking incubations at 37° in a water bath are used to determine the enzyme activity. The reaction takes place in 0.1 M potassium phosphate buffer, pH 7.8. The 1.0 mM NADPH is omitted from the blanks and the assay is started by addition of the substrate (final concentration 0.25 μM). An internal standard of 0.05 μM resorufin is recommended. The end point reaction is terminated by the addition of 2.0 ml cold acetone. The fluorescence of the resulting acetone/water mixture is measured by exciting at 510 nm and monitoring the fluorescence emission at 585 nm. Protein concentrations up to 0.20 mg/ml have been found to be linear in the author's laboratory. Ethoxyresorufin O-deethylase activity is inducible to over 20 fold (31-33). When highly induced enzyme activity is noted, lower protein concentrations and shorter time incubations should be run to confirm that the assay is still linear with protein concentration. The substrate and product have been commercially available from Molecular Probes Inc., 24750 Lawrence Road, Junction City, OR 97448. Alternatively, the substrate can be synthesized from the more readily available product resorufin by published procedures (31).

Reduced glutathione content

Work with bromobenzene and acetaminophen has developed the concept of glutathione depletion as a mechanism of toxicity (34-37). When 80% of hepatic glutathione is depleted by acetaminophen administration (35, 36), substantial increases in tissue covalent binding occur. Thus glutathione depletion is a useful toxicological theory to help explain the dose response thresholds. If a parent compound is metabolized to an intermediate which requires reduced glutathione for its further metabolism, transitory depressions in hepatic reduced glutathione may result.

A 20% homogenate of rat liver is prepared in 1.15% KC1 containing 0.02 M Tris-HC1, pH 7.5, 0.5 ml of the hepatic homogenate may be frozen and stored in a sealed test tube containing 8.5 ml of 0.012 M EDTA, pH 7.4, and 1.0 ml of 50% trichloroacetic acid. Before the liver homogenate is added, N_2 should be bubbled through the EDTA and trichloroacetic acid solution for 5 minutes to deoxygenate the solution (38). The deoxygenation procedure and the use of EDTA are needed to prevent the oxidation of the reduced glutathione (38). Samples should be frozen as quickly as possible and assayed soon after thawing. Once thawed, samples are centrifuged at 9,000 x g for 10 minutes. Two ml of the resulting supernatant are added to 4.0 ml of 0.4 M Tris-HCl, pH 8.9, in a new test tube. A 0.1 ml aliquot of 5,5'-dithiobis-(2-nitrobenzoic acid) (99 mg of this compound freshly dissolved in 25 ml of absolute methanol on the day of the assay) is added to the contents of the test tube and all three ingredients are mixed well. The resulting color is measured at 412 nm at room temperature five minutes after mixing. A no liver homogenate blank is used and internal standards of 100 and 200 μg of reduced glutathione are recommended. An excellent paper by Sedlak and Lindsay (38) describes the assay of reduced glutathione content.

Glutathione S-Transferase

The two most important pharmacologic effects related to glutathione S-transferase are enzyme induction and cofactor (glutathione) depletion. In rats, glutathione S-transferases occur in several different forms (about 6 or more) and the substrate specificity of these isoenzymes overlaps to a greater extent than does the cytochrome P-450 isoenzyme family (5, 6, 14). Three substrates frequently employed to assay its activity *in vitro* are 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-dichloro-4-nitrobenzene (DCNB) and 1,2-epoxy-3-(p-nitrophenoxy) propane (ENPP). The glutathione S-transferases can be induced about 2 to 3-fold, but in general these cytosolic enzymes are less responsibe than the cytochrome P-450 or UDP-glucuronyltransferase systems.

Oftentimes a compound will induce cytochrome P-450 and glutathione S-transferase in a coordinate fashion. The constitutive activity of hepatic glutathione S-transferase towards several of its substrates is much higher than the rate of catalysis by cytochrome P-450. In addition, as the ratio of liver cytosolic/microsomal protein is approximately 5/1, the liver has a large amount of glutathione S-transferase enzyme available as a detoxification pathway. Some nonenzymatic conjugation with glutathione may take place as well. This fact requires that a no tissue blank be used in assays for glutathione S-transferase. *In vivo* rat liver cytosolic glutathione concentration is approximately 5 mM.

The assays described here are taken from the paper of Habig et al. (14). The kinetic assays of glutathione S-transferase should be performed at 37°. Aqueous solubility is a problem for all three substrates and they are not used in truly saturating concentrations in the assay. This limits the time linearity. The stock solutions of the substrates CDNB (25.0 mM), DCNB (25.0 mM) and ENPP (20.0 mM) should be prepared in 100% ethanol, 100% ethanol and 50% ethanol/water respectively. Evaporative loss of the solvent may cause the substrate stock solutions to become too concentrated. This may cause precipitation of the substrate out of the assay buffer and interference with light transmittance in the spectrophotometer cuvette. If erratic tracings are encountered in the kinetic assay, the homogenity of the curvettes' contents should be visually checked. Assay conditions are as presented in Table I. A lower glutathione contentration is used for CDNB than with

the other two substrates. The time and protein linearity of these assays are limited (*e.g.*, 2 to 5 minutes and 0.003 mg protein from rat hepatic high speed supernatant for CDNB), in part because of the high specific activity of the transferases. CDNB is the easiest glutathione S transferase substrate to use, while DCNB is a more difficult substrate to use. Determining the activity of glutathione S-transferase towards ENPP is even more difficult as the enzyme activity is low, the substrate is of low water solubility, the extinction coefficient unfavorable, and the required protein concentration is higher (increased turbidity tends to unbalance the light absorption in the two cuvettes of a dual beam spectrophotometers).

UDP-Glucuronyltransferase

Multiple forms of UDP-glucuronyltransferase (GT) exist (7, 8). Wishart (8) has distinguished between 'late fetal' GT (representative substrate p-nitrophenol) and 'neonatal' GT (representative substrate phenolphthalein) forms on the basis of gluccorticoid induction, zenobiotic induction, smooth/rough endoplasmic reticulum ratios, ontogeny and substrate specificity. The GT isoenzyme forms take their name from the developmental time in which their enzyme activity is at its peak. Only late fetal GT is highly inducible; neonatal GT is not. Late fetal GT is highly inducible by cytochrome P-448 type inducers such as 3-methylcholanthene, and thus coordinate induction of cytochrome P-448 and late fetal GT can occur. The late fetal GT form tends to conjugate smaller, more planar structures while neonatal GT substrates can be large, bulky molecules. In the neonatal GT group are several substrates of physiologic or toxicologic importance such as bilirubin, testosterone, estrogen, chloramphenicol and morphine.

Microsomes are activated for assay of GT by homogenization with 0.2% Triton X-100 (39). This treatment increases the activity of the glucuronyltransferases many fold and makes the enzyme activity much easier to determine. Different investigators use different detergents to activate GT activity, and some investigators will run a series of detergent concentrations with each microsomal sample and use the highest enzyme activity observed as the true GT rate.

In 0.15 M Tris-HC1 buffer, pH 7.4, containing 0.01M $MgCl_2$, assays of late fetal and neonatal GT are performed using p-nitrophenol and phenolphthalein as the respective substrates. Uridine diphosphate glucuronic acid (1.4 mM final concentration) is the cofactor used in the 10 minute end point 37° C incubations. The cofactor is omitted from blanks and the enzyme assay is started by the addition of the microsomal protein. P-nitrophenol stock solutions can be prepared in water, but phenolphtholein should be disolved in 50% ethanol/50% water. The final substrate concentration with phenolphthalein is only 0.2 mM (40) compared to 0.8 mM for p-nitrophenol. Both assays are almost universally done by substrate disappearance even though product appearance assays would seem to be appropriate for both substrates (41). P-nitrophenol glucuronide and phenolphthalein glucuronide are both commercially available to use as internal standards. The substrate disappearance reactions are terminated by adding 7 or 14 ml of 0.2 M glycine buffer, pH 10.4, to the GT assays for phenolphthalein and p-nitrophenol, respectively. The glycine buffer stops the enzyme reaction, maintains a basic pH required for maximal color development, and dilutes the substrate to a concentration for which spectrophotometric detection is linear. With late fetal GT (p-nitrophenol) large increases in enzyme activity are possible, so the recommended protein and time linearity values may have to be reduced to maintain linear conditions with highly induced microsomes. Wavelengths of 405 and 553 nm are used to determine the concentration of p-nitrophenol and phenolphthalein left after the enzyme assay incubations are completed. Triton X-100 treated microsomes may be stored frozen and assayed at the convenience of the investigator.

General comments

All enzyme assays described in this review should be done at 37° C. Kinetic assays should use cuvette stirrers if possible and end point assays should be shaken in a constant tempera-

ture water bath. Subcellular fractions should be prepared by conventional centrifugation procedures. Whole liver homogenates (glutathione assay), microsomes and the 105,000 x g high speed supernantant are the fractions needed for these 10 hepatic parameters.

A spectrophotofluorometer is required to determine ethoxyresorufin O-deethylase activity. It this instrumentation is not available, the radiometric benzo[α]pyrene monooxygenase assay is the best alternative. No good spectrophotometric technique to assay hepatic microsomal cytochrome P-448 mediated enzyme activity has been described. Some of these assay methods require a dual wavelength spectrophotometer (NADPH-cytochrome C reductase, the glutathione S-transferases and cytochrome P-450 determinations).

Conclusion

For reasons of brevity, several important biotransformation pathways such as sulfation, acetylation, methylation, dehalogenation, dehydrohalogenation and epoxide hydration are omitted from this methodological review. From the information presented in Table I and the discussion in the text, investigators should be able to successfully implement this hepatic toxification/detoxification parameter test battery or any elements in it more easily than they could by consulting the primary literature alone. For some of these assays neither Methods in Enzymology, Advances in Enzymology or the primary literature give totally satisfactory details of the assay. In other cases, several popular variations of a particular assay or many different versions of the same assay are publisched, so the choice of which method variation to emply can be difficult for investigators inexperienced in this field. Representative values for the rat hepatic toxification/detoxification parameters are presented in Table II to further assist investigators in initiating studies in this important area of pharmacology and toxicology.

Abbreviations

KP, potassium phosphate; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4 nitrobenzene; ENPP, 1,2-epoxy-3-(p-nitrophenoxy) propane.

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Table II. Representative Rat Hepatic Parameters[a,b] (nmole/mg protein/min)

	Male	Female	Day 14 Pregnant Female
NADPH-cytochrome C reductase	120.	107.	116.
Cytochrome P-450 (nmole/mg protein)	1.06	0.85	0.73
Aminopyrine N-demethylase	—	3.94	2.61
Ethoxyresorufin 0-deethylase	.022	—	0.014
Glutathione (nmol/mg protein)	—	333.	306.
Glutathione S-transferase towards			
1-chloro-2,4-dinitrobenzene	—	—	2,370.
1,2-dichloro-4 nitrobenzene	—	—	34.
1,2-epoxy-3-(p-nitrophenoxy) propane	—	—	71.
UDP-Glucuronyltransferase towards			
p-nitrophenol[c]	33.0	40.8	19.4
phenolphthalein[c]	—	16.8	9.5

[a]Values are representative measurements made during different investigations in the author's laboratory. Values can not be fairly compared to one another as they may be measurements performed on different days.
[b]Adapted from (42).
[c]Enzyme activity after Triton X-100 detergent activation. Non-activated GT activity is about 1/10 these values.

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Disclaimer

This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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